NAVAL HEALTH RESEARCH CENTER

THE QUANTITATIVE IMMUNOPHENOTYPE
(QuIP) IS CHARACTERISTIC OF INDIVIDUALS

D. Redelman
W. Ensign
D. Roberts

20040303 183

Report No. 01-03

THE QUANTITATIVE IMMUNOPHENOTYPE (QuIP) IS

CHARACTERISTIC OF INDIVIDUALS

Doug Redelman[2]

Wayne Ensign[1]

Don Roberts[1]

[1] Naval Health Research Center
P. O/ Box 85122
San Diego, California 92186-5122

and

[2] Sierra Cytometry & UNR Cytometry Center
Reno, Nevada

THE QUANTITATIVE IMMUNOPHENOTYPE (QuIP) IS CHARACTERISTIC OF INDIVIDUALS

Doug Redelman

Sierra Cytometry **& UNR Cytometry Center**

Reno, NV

Wayne Ensign and Don Roberts

Naval Health Research Center

San Diego, CA

Abstract

The clusters of differentiation (CD) molecules used to identify lineages of cells often have important functional activities. Furthermore, the numbers of CD molecules expressed per cell can reflect the status of cell lineages. For example, B cells with abnormal numbers of CD19 per cell have altered capacities to produce antibody responses. Therefore, the quantitative immunophenotype (QuIP), *i.e.*, both the numbers of cells in various populations and the numbers of CD molecules expressed per cell, can provide additional information about the functional capacity of the immune system. In order to examine the variations in QuIP among individuals and within individuals over time, blood samples from a cohort of Marines were tested three times over a two month period before and after various phases of strenuous advanced field training exercises. Samples were examined *via* a standard hematology analyzer to obtain complete blood counts and flow cytometrically with a panel of antibodies. The relative numbers of CD molecules per cell were estimated by using standard beads with known numbers of fluorochrome molecules per bead or with known antigen binding capacities per bead. Within individuals, the relative percentages of cell lineages and the numbers of CD molecules expressed per cell changed very little over two months although there was substantial variation among individuals at each time. Thus, measuring the QuIP can provide additional and potentially valuable information about the status of the immune system.

Introduction

Intense physical exercise, *e.g.*, a marathon run, is known to cause changes in leukocytes (*e.g.*, Gabriel, *et al.*, 1998). The effects on the immune system of more prolonged strenuous exercise, as typified by various types of military training programs, have been less well characterized. Therefore, the present study was undertaken to monitor a cohort of Marines during training exercises near sea level and at moderate elevation in winter climate conditions. These Marines, who had been in the military for a year or more, were based at 29 Palms in Southern California. At the beginning of February, 2000, they were transported to the Mountain Warfare Training Center (MWTC) at ~7,000ft in the Eastern Sierra Nevada near Bridgeport, CA. During their time at the MWTC (~3 weeks), they participated in winter training exercises mostly at elevations of ~10,000ft. They were then returned to 29 Palms where they continued normal training, e.g., daily runs, etc. For this study, blood samples were collected upon arrival at the MWTC, at the completion of the MWTC training and then ~3-4 weeks later at 29 Palms. Although several other types of tests were performed on these blood samples, the only data to be reported here are the results differential cell counts and immunophenotyping with a panel of monoclonal antibodies. The results show that among the 40+ individuals that were examined, there was substantial variation in the absolute or relative numbers of leukocyte subpopulations and in the quantitative expression of CD molecules on T cells, B cells and monocytes. On the other hand, these properties changed much less within individuals and there was no overall change that could be ascribed to the effects of prolonged strenuous exercise at 7-10,000 ft.

Materials and Methods

Blood samples and labeling with antibodies -- Blood samples were collected, after informed consent, in EDTA-anticoagulated Vacutainer tubes, chilled and transported from the MWTC or from 29 Palms to Reno. Aliquots were removed for complete blood counts (CBC) at the Reno Laboratory Corporation of America clinical lab.

Description	Cat No.	Clone	Ig	Lot No.
FITC-anti-CD2 (F/P = 6.125)	30054X	RPA-2.10	M IgG1 K	M040696
FITC-anti-CD3 (F/P = 5.91)	30104X	UCHT1	M IgG1 K	M041533
PE-anti-CD4	30155X	RPA-T4	M IgG1 K	M039902
CyC-anti-CD8	33298X	HIT8a	M IgG1 K	M040067
CyC-anti-CD11b	30458X	ICRF44(44)	M IgG1 K	M044581
PE-anti-CD14	30545X	M5E2	M IgG2a K	M043400
FITC-anti-CD15	30574X	HI98	M IgM K	M027053
PE-anti-CD16	30625X	3G8	M IgG1 K	M041541
PE-anti-CD19	30655X	HIB19	M IgG1 K	M043401
FITC-anti-CD25 (F/P = 4.6)	30794X	M-A251	M IgG1 K	M042772
CyC-anti-CD45	31258X	HI30	M IgG1 K	M048151
CyC-anti-CD56	31668X	B159	M IgG1 K	M040686
FITC-Mo-IgM (k) (TNP)	33064X	G155-228	M IgM K	M043586
PE-Mo-IgG2a (TNP)	33035X	G155-178	M IgG2a K	M042623
CyC-Mo-IgG1 (k) (?)	33818X	MOPC-21	M IgG1 K	M041503

Table 1. Monoclonal antibodies — All listed antibodies were obtained from PharMingen and the same lots were used for all tests.

Monoclonal antibodies, as listed in Table 1, were mixed at equal volumes
according to the combinations listed in Table 2 and the mixed antibodies were
then added to 200µl aliquots of blood.

Antibody Combinations

	Fluorescein (FITC)	R-Phycoerythrin (PE)	CyChrome (CyC)
1.	anti-CD3	anti-CD14	anti-CD45
2.	anti-CD3	anti-CD4	anti-CD8
3.	anti-CD25	anti-CD4	anti-CD8
4.	anti-CD3	anti-CD16	anti-CD56
5.	anti-CD2	anti-CD4	anti-CD8
6.	anti-CD3	anti-CD19	anti-CD45
7.	anti-CD15	anti-CD14	anti-CD11b
8.	IgM	IgG2a	IgG1

Table 2. Antibody Combinations -- Cells labeled with combination #1 were
tested with TruCount Beads.

After incubation in the cold, FACSLyse solution was added to lyse erythrocytes and
fix the cells, the cells were washed by centrifugation and resuspended in FACS
Buffer with 1% fetal bovine serum. Labeled cells were kept chilled and light
protected until flow cytometric examination which occurred within 24-48 hr of
labeling. All samples were processed and examined in sets according to the
antibody combinations listed in Table 2.

Flow cytometric examinations -- All flow cytometry was done using a four-color Coulter XL/MCL with optical filters of 525nm (FL1, fluorescein), 575nm (FL2, PE), 620nm (FL3, PE-Texas Red or propidium iodide) and 675nm (FL4, PE-Cy5) that was operated with Coulter System II acquisition software. Color compensation was set using cells that had been individually labeled. Data files of 20,000 events were collected and stored in list mode using the MCL.

Analyses of data -- Data files were analyzed using SuperCyt Analyst software (Sierra Cytometry) using PhenoGates to define multiple populations of interest. Each population was defined using upper and lower boundaries in one or more parameters as needed. In the present experiments, as many as 12 separate populations were defined for analysis. The analyses of these populations, including the numbers of cells and the mean fluorescence intensity (MFI), were done in batch mode with results saved to database files. Secondary analyses were performed with standard spreadsheet software such as Microsoft Excel. MFI values were converted to molecules of equivalent soluble fluorochrome (MESF) using standard beads with known MESF values as more fully described in the accompanying presentation. The binding of fluorescein-labeled antibodies was converted to molecules of antibody bound using the fluorescein:protein ratios provided by the manufacturer. Although the fluorochrome substitution of the PE and PE-Cy5 labeled antibodies was not exactly specified, the data in the accompanying presentation suggest that the PE-labeled antibodies most likely had ~1PE per IgG.

Results and Discussion

This project was designed to examine the effects of prolonged strenuous physical activity on the immune response. Absolute and relative cell numbers were determined as is commonly done in flow cytometric analyses. In addition, since potentially subtle changes in cell properties were of interest, the quantitative expression of differentiation molecules was also measured.

Absolute and differential leukocyte counts

Total Lymphocyte counts -- Among the subjects in the study, there was substantial variation in the absolute numbers of total leukocytes, leukocyte subsets and in the relative numbers of cell populations. At the Pre-MWTC test, the mean lymphocyte count was 1,908/µl (range 931-3,590), at the Post-MWTC test, the mean lymphocyte count was 2,108/µl (range 1,072-3,120) and at the Final test the mean lymphocyte count was 2,007/µl (range 982-5,264). The total lymphocyte concentrations in the Pre-MWTC and Final samples are depicted in Figure 1 in which individuals are shown at the two time points. There is a slight increase with time as shown in the mean values but the scatter of the data illustrate that total lymphocyte counts varied among individuals and within individuals over the 7 week time course of these comparisons. The changes in the numbers of circulating lymphocytes were not unexpected since several of the subjects reported upper respiratory tract infections during this interval.

Total Lymphocytes


$y = 1.0269x$
$R^2 = 0.1051$
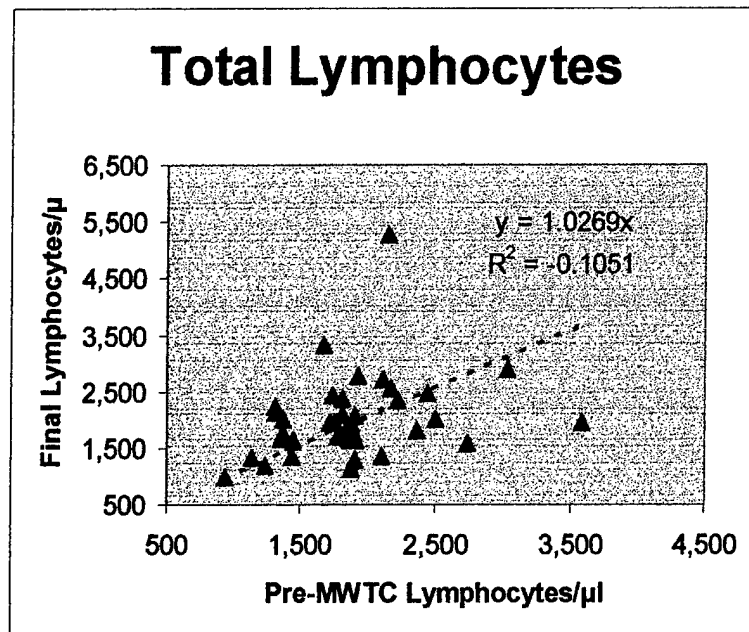
Figure 1. Total lymphocyte counts — Lymphocytes were identified on the basis of CD45 expression and light scatter properties and enumerated using TruCount beads.

Composition of lymphocytes -- The relative numbers of the principal lymphocyte subsets are depicted in Figure 2. The composition of lymphocytes among these subjects varied substantially as shown. Within individuals, there was also variation in the relative percentages of T cells and natural killer (NK) cells over the time that was examined. On the other hand, the relative numbers of B cells, although widely variable among subjects from ~5%-25%, changed very little within individuals.
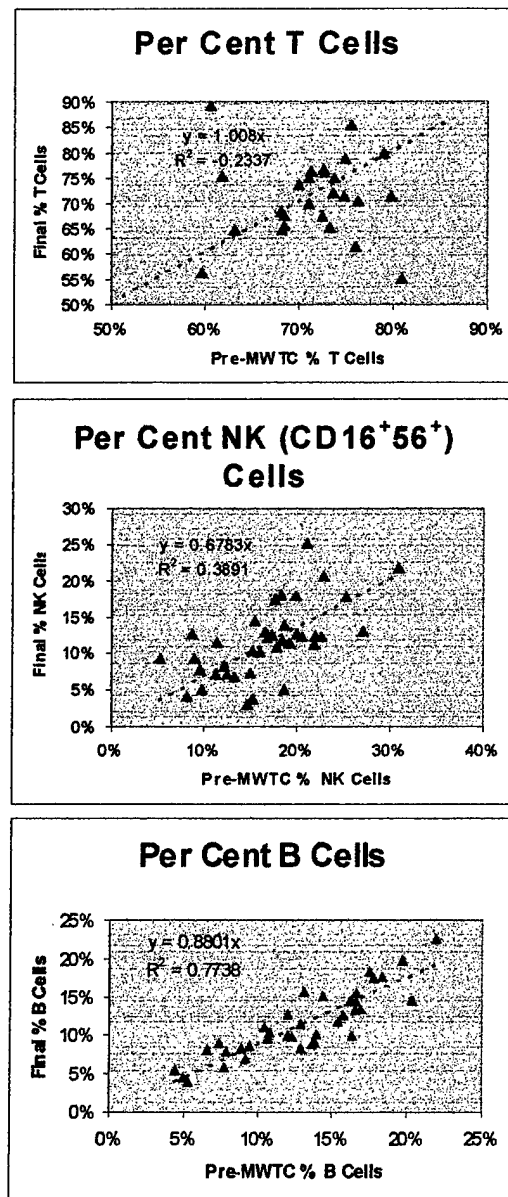
Figure 2. Lymphocyte Composition — The relative numbers of T cells (CD3^{+}), natural killer cells (NK, CD3[-]16^{+}56^{+}) and B cells (CD19^{+}) are expressed as percentages of the total lymphocytes.

Composition of T cells -- Within the total T cell population, the relative numbers of T helper (Th, CD3+4+8-), T cytotoxic/suppressor (Tc/s, CD3+4-8+) and T double negative (Tdn, CD3+4-8-; Lanier, *et al.*, 1986) cells remained much more consistent within individuals as shown in Figure 3.
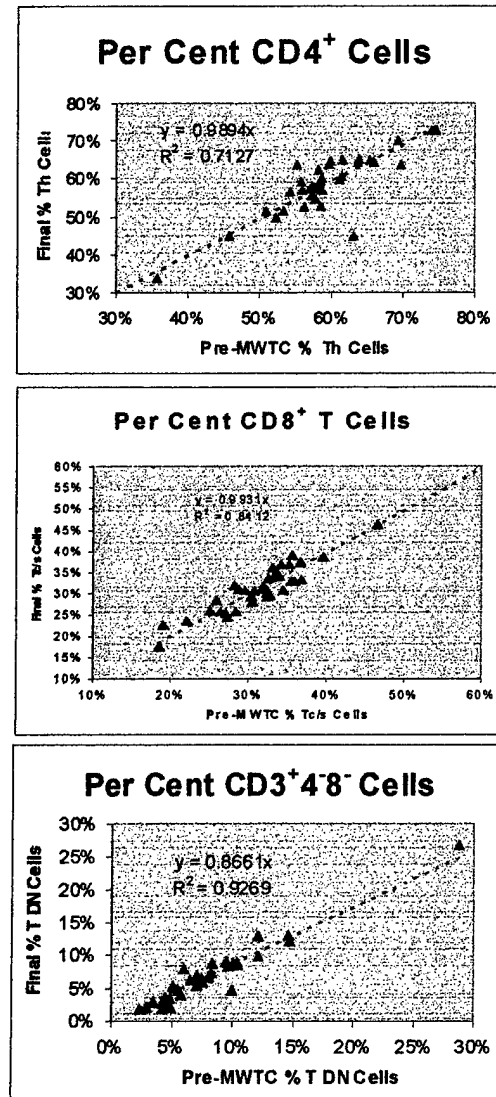

Figure 3. Composition of T Cells — The principal T cell subsets defined with antibody combination 2 in Table 2 are depicted as T cell percentages in the initial and final tests. The number of T cells apparently expressing both CD4 and CD8 was negligible and is not shown.

# Quantitative Expression of Differentiation Antigens

The number of CD antigens per cell is expressed as the MESF of the PE or CyChrome labeled antibodies or as molecules of fluorescein-IgG antibodies bound per cell. The PE labeled antibodies most likely had ~1 PE per IgG as outlined in the accompanying presentation. The substitution ratios of the CyChrome labeled antibodies cannot be estimated readily.

CD19 on B cells -- The CD19 molecule occurs on B cells where it is part of the antigen receptor complex (Carter, *et al.*, 1988). Figure 4 depicts the expression of CD19 on B cells at the beginning and end of the study interval. As shown, CD19 varied from ~17,000-27,000 MESF of PE-anti-CD19 among the individuals tested. The data in Figure 4 also show that the CD19 expressed per B cell was relatively consistent within individuals. In data not shown, there was no apparent relationship between the absolute or relative number of B cells and the amount of CD19 expressed per cell. Studies of human and animal B cells have demonstrated that relatively small changes in the amount of CD19 expressed per cell can affect the activation threshold of the cells (Carter & Fearon, 1992). For example, mice deficient in CD19 expression are also deficient in their response to protein antigens (Rickert, *et al.*, 1995; Sato, *et al.*, 1996). Furthermore, mice that over-express CD19 by as little as 25% become hyper-responsive with elevated levels of anti-DNA and rheumatoid auto-antibodies (Sato, *et al.*, 1996). Thus, it seems likely that some of the normal healthy donors have levels of CD19 that are either elevated or depressed, depending upon what is defined as the "normal" level of CD19 expression.
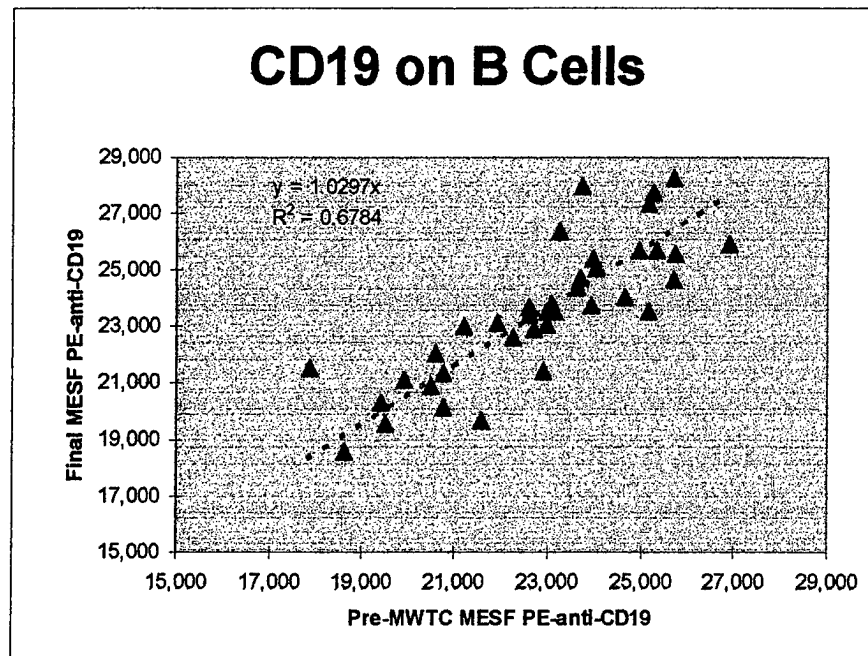
CD19 on B Cells

Figure 4. Expression of CD19 on B Cells — The number of CD19 molecules on B cells is expressed as the MESF of PE-anti-CD19 bound per cell.

CD14 on Monocytes -- The CD14 molecule on monocytes is lipid linked (Haziot, *et al.*, 1988) and forms part of the LPS receptor (Goyert, *et al.*, 1988). In patients with septicemia, it has been shown that the prognosis is poor if the number of CD14 monocytes is reduced (Jason, *et al.*, 1999) or if the CD14 expression on monocytes is low Heinzelmann, *et al.*, 1996). It has been reported that human monocytes "normally" express ~110,000 CD14 molecules per cell (Antal-Szalmas, *et al.*, 1997) which would be consistent with cells binding ~50,000-60,000 MESF of PE-anti-CD14 (assuming ~1 PE per IgG). Unexpectedly, it was found that the amount of CD14 expressed on the monocytes of these normal subjects varied by approximately an order of magnitude. However, as noted with CD19 expression, the comparisons in Figure 5 showed overall consistency of CD14 expression within these normal individuals.

CD14 on Monocytes

$y = 1.0784x$
$R^2 = 0.7343$

(scatter plot: Final MESF PE-anti-CD14 vs. Pre-MWTC MESF PE-anti-CD14)
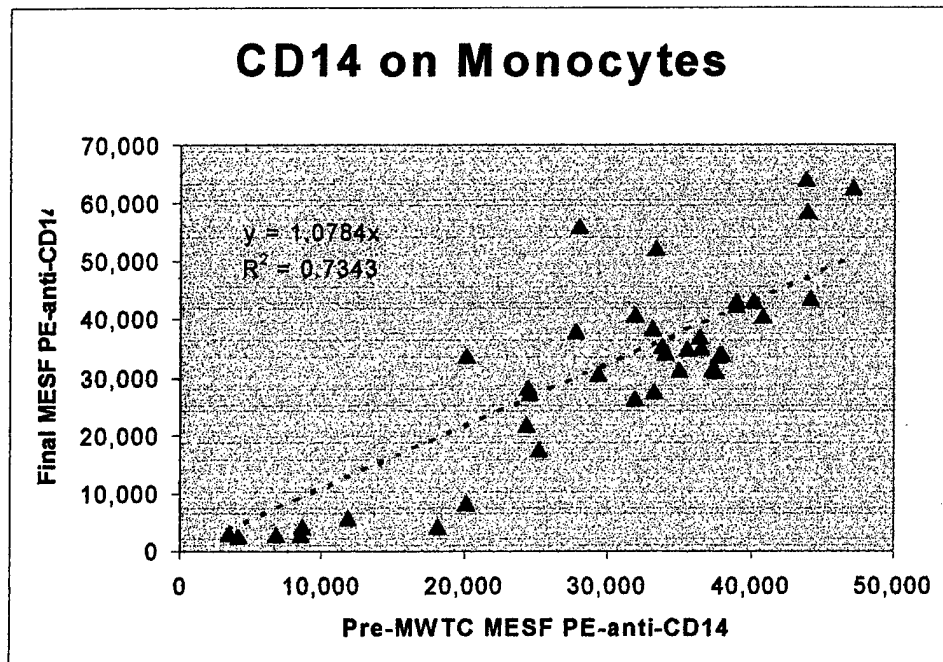
Figure 5. Expression of CD14 on Monocytes — The number of CD14 molecules on monocytes is expressed as the MESF of PE-anti-CD14 bound per cell.

Expression of CD3 on T Cells -- The amount of CD3 expressed on T cells varied among individuals and within individuals on the Th, Tc/s or Tdn subsets of T cells. As previously reported by Ginaldi, *et al.*, (1996), the CD4+ cells expressed higher amounts of CD3 than did CD8+ T cells. In addition, we noted that the Tdn cells had the highest expression of CD3. These data from the Pre-MWTC test are summarized in Table 3 and depicted for all the subjects in Figure 6. The individual data shown in Figure 6 illustrate that the levels of CD3 expression on CD4 and CD8 cells were directly related whereas the CD3 on Tdn cells appeared to vary independently. This observation would be consistent with Tdn cells comprising a separate differentiation lineage.

Molecules FITC-anti-CD3 Bound to T Cell Subsets

|	CD4	CD8	T DN
Min =	26,358	19,535	22,361
Max =	42,143	31,305	58,516
Mean =	32,613	25,608	40,229
StdDev =	5,913	4,637	10,252

Table 3. CD3 on T Cells — Data from the Pre-MWTC tests were used to calculate the mean number of FITC-anti-CD3 antibodies bound per cell in each of the three principal T cell subsets.
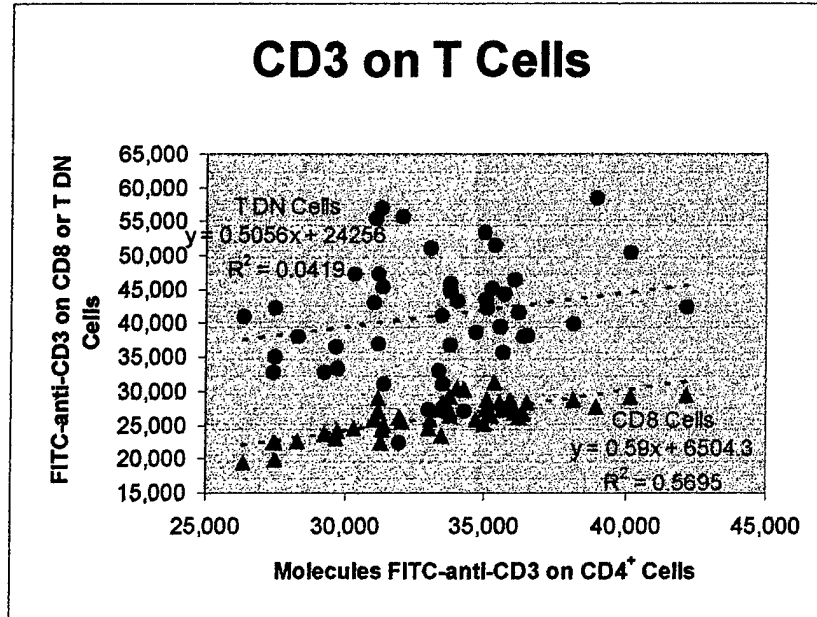
Figure 6. Expression of CD3 on T Cells — The expression of CD3 on CD8 or on Tdn cells is plotted as a function of the amount of CD3 on the CD4 cells from that individual.

Expression of CD4 and CD8 -- Although the amount of CD4 on T helper cells has been considered relatively invariant and has been used as an internal biological standard (Hultin, *et al.*, 1998), we found that CD4 expression varied among individuals. The data in Figure 7 show that the amount of CD4 expressed per cell did not appear to be related to the number of CD4 cells or to the amount of CD8 expressed on cells from the same donor.
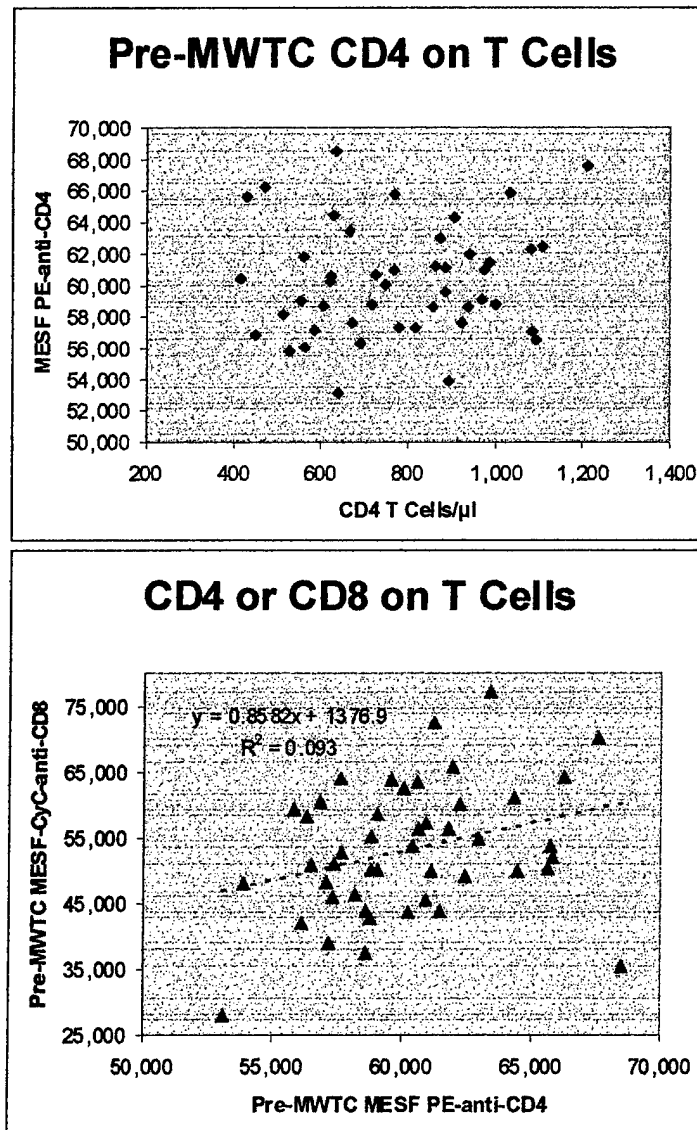
Figure 7. CD4 or CD8 on T Cells — In the upper panel, the expression of CD4 is compared with the CD4 T cell counts. In the lower panel, the MESF of PE-anti-CD4 or of CyC-anti-CD8 antibodies bound per cell in the Pre-MWTC test are compared.

Although not invariant, the variation in CD4 expression was considerably less than that of CD8 in the same donors. When the same donors were compared over time, both molecules also varied in expression as shown in Figure 8.
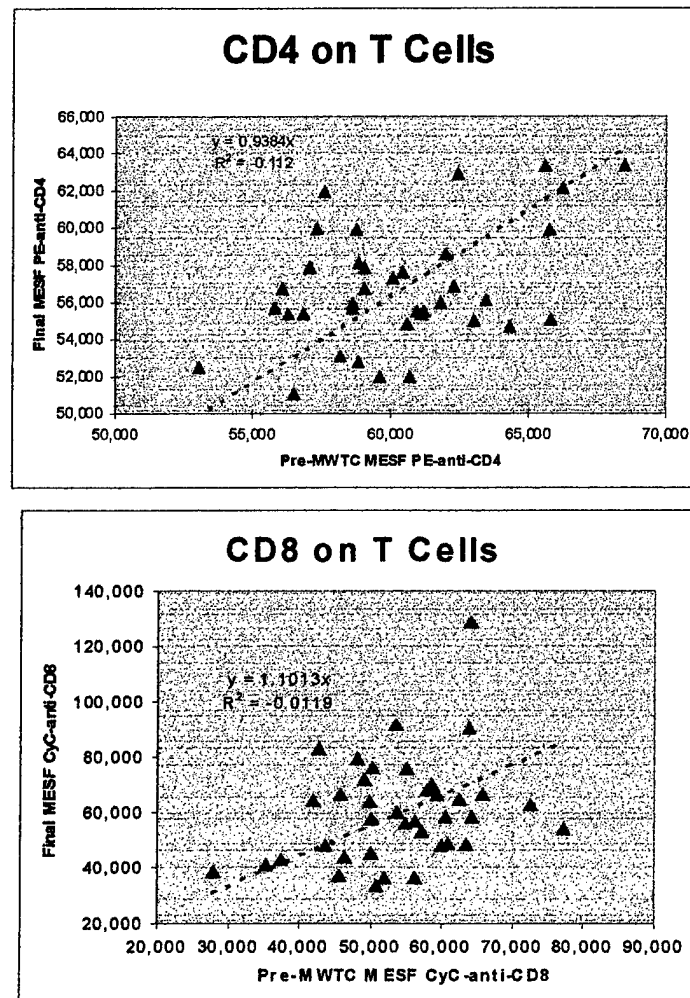

Figure 8. Expression of CD4 or CD8 — The MESF of PE-anti-CD4 or of CyC-anti-CD8 bound per cell in the Pre-MWTC and final tests are compared.

Expression of CD2 -- T cells could be further subdivided on the basis of CD2 expression. It has been previously demonstrated that CD2 expression is substantially elevated during T cell activation (Redelman, 1987). After activation with Il-2 present (Roth, 1994), resting "memory" (CD45RA-CD45RO+) T cells express higher levels of CD2 than do CD45RA+ cells (Prince, *et al.*, 1992). Figure 9 depicts the relative fluorescence intensities of FITC-anti-CD2 on CD4+ or CD8+ cells from two donors. In these typical donors, CD2 expression was bimodal with varying proportions of cells in the high and low peaks corresponding to memory and naive cells, respectively. In contrast to many of the differentiation antigens, there was little variation in the level of expression of CD2 within these two peaks. However, there was considerable variation in the proportions of CD4 or CD8 cells having the two levels of CD2.

# CD2 on CD8⁺ T Cells   CD2 on CD4⁺ T Cells
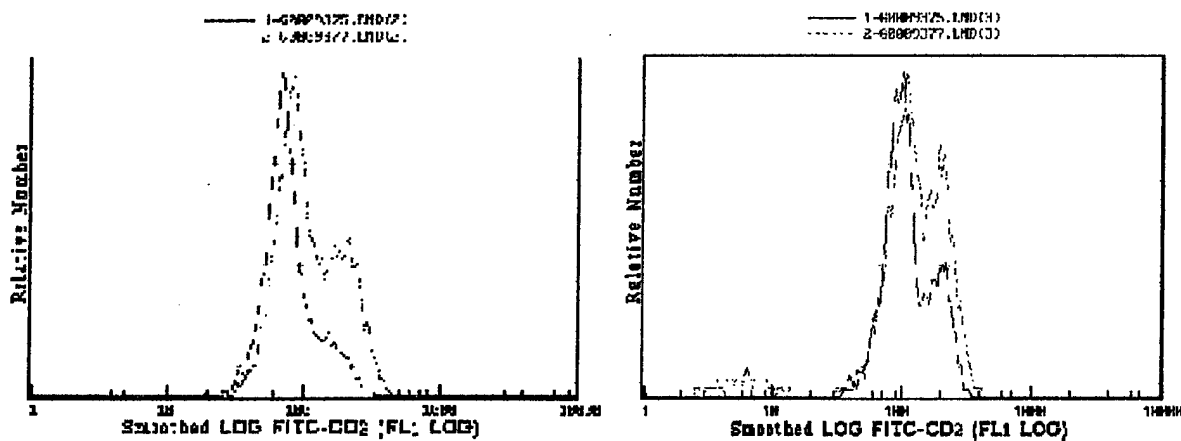
Figure 9. CD2 on CD4 or CD8 Cells — Relative fluorescence intensities of FITC-anti-CD2 labeling of CD4 or CD8 cells are shown.

As shown in Figure 10, the fraction of CD4+ or CD8+ cells with the higher level of CD2, i.e., "memory" cells, varied from ~10%-80%. This wide range of variation was unexpected in these young subjects since higher proportions of memory cells are characteristically associated with more aged populations (Ginaldi, *et al.*, 2000). Within individuals, the fraction of CD8+ memory cells was typically higher than that of CD4 cells. The data depicted in the panels of Figure 10 also illustrate that the relative proportions of CD4+ or CD8+ cells with the two levels of CD2 expression remained relatively constant over the period examined.
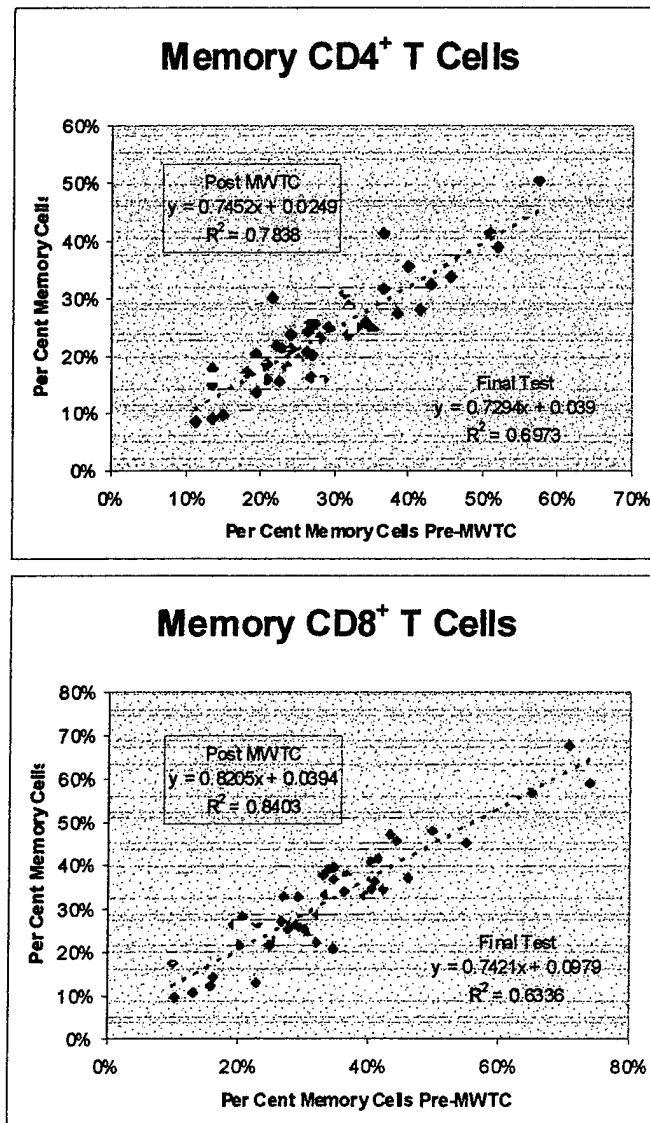

Figure 10. Proportions of Memory T Cells — The percentages of CD4 or of CD8 cells with high expression of CD2 are compared among the three tests.

The amounts of CD4 or CD8 on naive and memory cells were compared as depicted in Figure 11. Those data show that CD4 was expressed at slightly higher levels on the subset with higher CD2 whereas CD8 was comparably expressed on cells with either level of CD2.

Expression of CD4

$y = 1.1672x$

$R^2 = 0.5894$

Expression of CD8

$y = 0.9915x$

$R^2 = 0.4575$
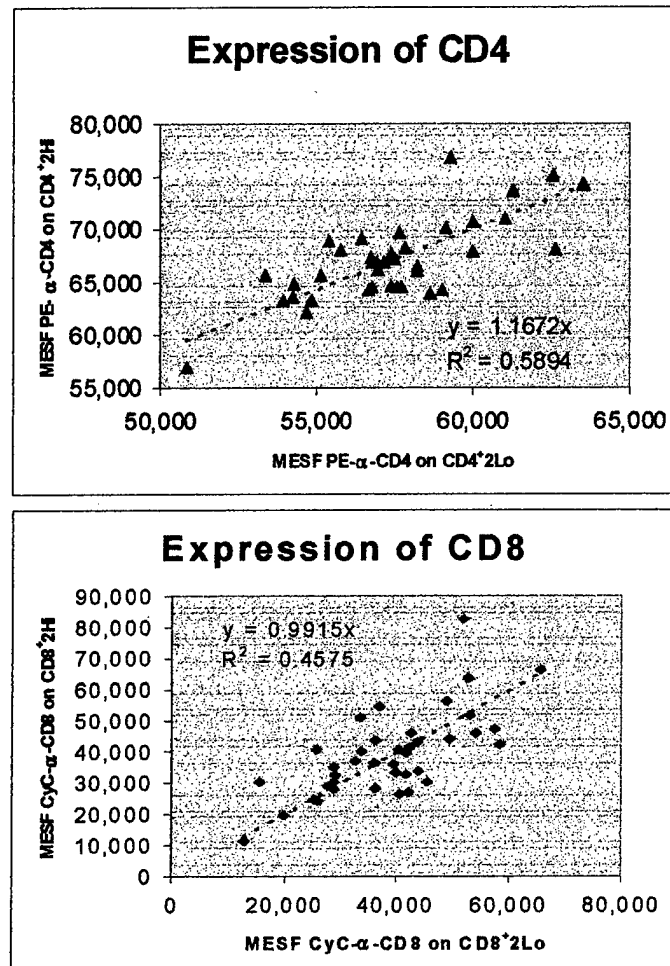
Figure 11. Expression of CD4 or CD8 on Naive or Memory Cells — CD4 was expressed at slightly higher levels on memory cells (upper panel) whereas CD8 was comparably expressed on naive and memory cells (lower panel).

Finally, although CD2 and CD3 expression were not examined simultaneously, it was noted that the level of CD3 expression on CD4+ or CD8+ cells was slightly lower in individuals with greater fractions of cells in the higher CD2 distribution as shown in Figure 12.
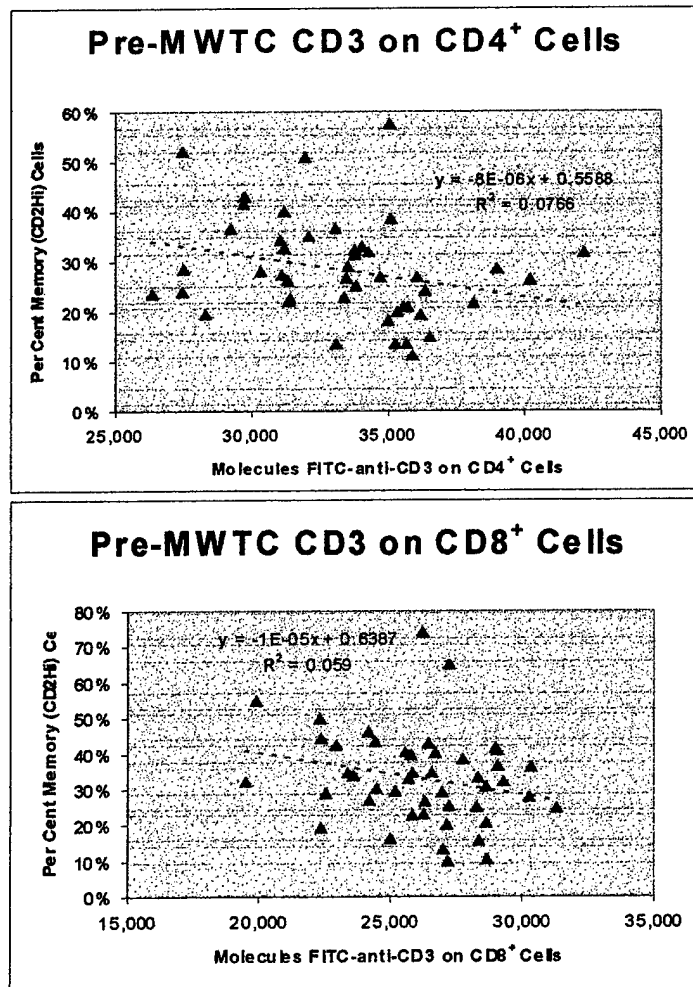
Figure 12. CD3 Expression *vs.* Per Cent Memory Cells — Although the correlations are low, the amount of CD3 expressed on CD4 or CD8 cells appeared to be lower as the fraction of memory cells identified with high CD2 expression increased.

Thus, in summary, CD3 was variably expressed among individuals and among subsets of T cells with expression lowest on CD8, intermediate on CD4 and highest on Tdn cells. It also appeared that CD3 was expressed at slightly lower levels on

memory cells that were identified as having higher levels of CD2. Although the relationships of CD3 expression on T cell subsets were consistent, CD3 expression did vary within individuals over the period of this study. CD4 and CD8 were also expressed variably among individuals but were more consistently expressed over time. CD8 expression did not appear related to CD2 expression whereas CD4 expression was higher on memory cells with higher CD2. Finally, the proportions of CD4 and CD8 cells with higher levels of CD2, *i.e.*, memory cells, were highly variable among individuals but remained consistent within individuals.

CD45 Expression -- Labeling with anti-CD45 has been widely used to distinguish leukocytes from irrelevant materials in lysed whole blood preparations (e.g., Borowitz, *et al.*, 1993). However, it was noted in the present experiments that the levels of CD45 expression varied more than expected. The data depicted in Figure 13 illustrate that CD45 expression varied substantially on lymphocytes and monocytes but relatively little on granulocytes.
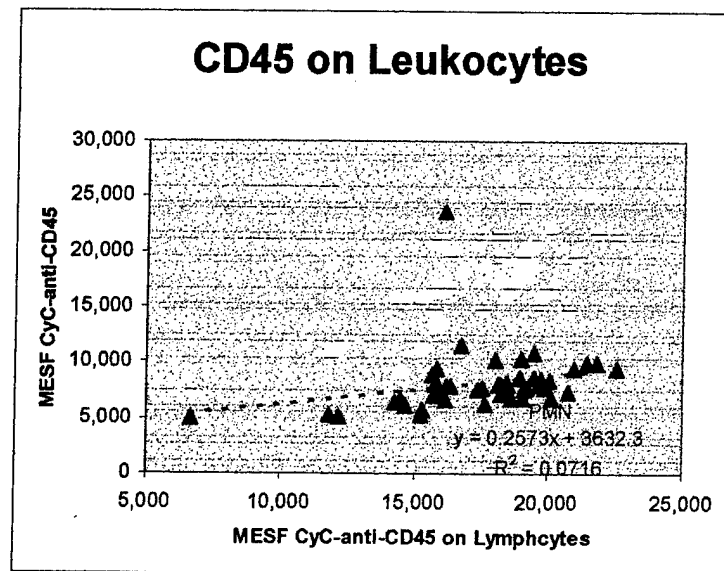
Figure 13. CD45 on Leukocytes — CD45 expression on granulocytes or on monocytes are compared with CD45 expression on the lymphocytes from the same donor in the Pre-MWTC test.

Figure 14 illustrates that individuals tended to be consistent in having high or low levels of CD45 expressed.

CD45 on Lymphocytes

$y = 0.9309x - 1210.7$
$R^2 = 0.3188$

CD45 on Monocytes

$y = 1.1533x - 1641.6$
$R^2 = 0.4835$
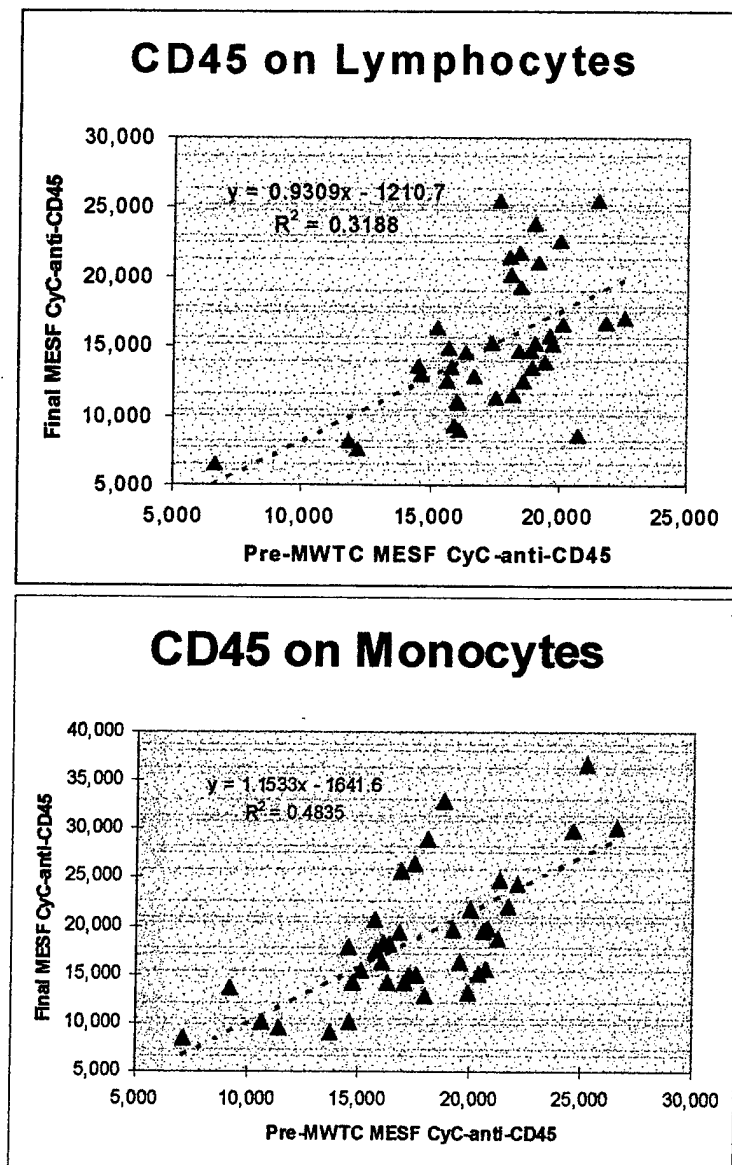
Figure 14. CD45 on Lymphocytes and Monocytes — The expression of CD45 on lymphocytes and monocytes in the Pre-MWTC and final tests are compared.

CD11b expression -- CD11b is expressed on activated granulocytes, on monocytes, on most NK cells and on CD8+ T cells with suppressor activity (Gane, et al., 1992). The data depicted in Figure 15 compare the amount of CD11b expressed on the monocytes examined in the three tests. These data illustrate that CD11b on monocytes varied among individuals over approximately a two-fold range and to a slightly lesser extent within individuals over time.
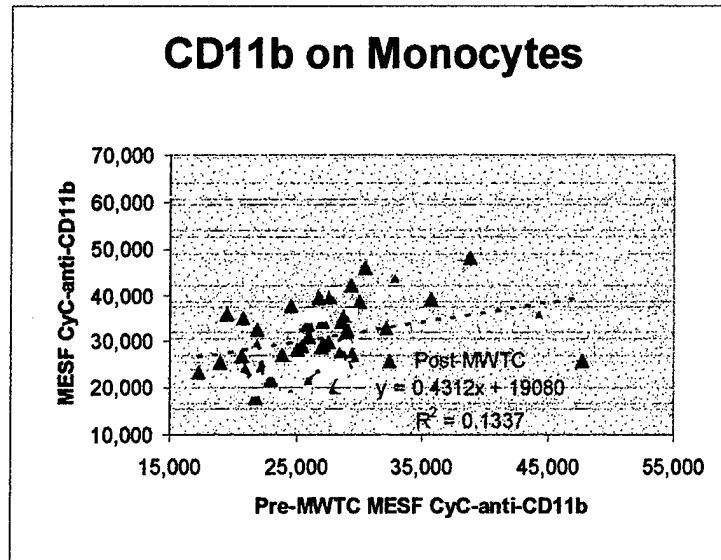
Figure 15. CD11b on Monocytes — The expression of CD11b on the monocytes examined from the Post-MWTC and the Final tests are compared with the amount expressed in the Pre-MWTC test.
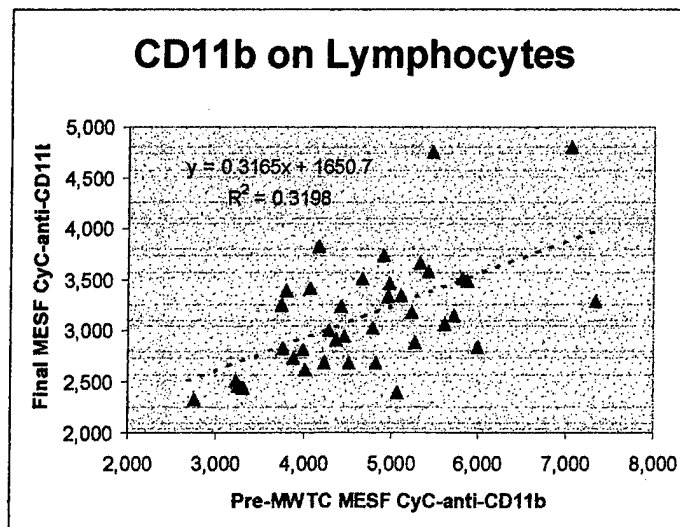
Figure 16. CD11b on Lymphocytes — The expression of CD11b on lymphocytes examined in the Pre-MWTC and Final tests are compared.

Figure 16 compares the CD11b on lymphocytes examined in the Pre-MWTC and Final tests. CD11b on lymphocytes also varied over a two-fold range of expression but was more consistent within individuals over time. The current tests did not permit one to distinguish the CD11b on CD8 suppressor T cells from that on NK cells. Thus, it is possible that CD11b could be expressed differently on those populations.

Summary and Conclusions

Among the present group of subjects, three weeks of strenuous military winter training at moderate elevation did not produce detectable overall effects on the composition or measured properties of T cells, B cells and monocytes. However, it was noted that these healthy and fit age and sex-matched subjects varied widely in the absolute and relative numbers of lymphocyte subsets. Furthermore, there was also wide variation in the expression of differentiation molecules such as CD19 and CD14 and in the relative proportions of T cells with a memory phenotype. Although these properties varied widely among the subjects, there was much less variation within subjects over the course of this study. Thus, differing levels of expression of CD molecules may comprise relatively stable characteristics of individuals.

References

Antal-Szalmas P, Strijp JA, Weersink AJ, Verhoef J, Van Kessel KP. 1997. Quantitation of surface CD14 on human monocytes and neutrophils. **J Leukoc Biol 61**(6):721-8.

Borowitz MJ, Guenther KL, Shults KE, Stelzer GT. 1993. Immunophenotyping of acute leukemia by flow cytometric analysis. Use of CD45 and right-angle light scatter to gate on leukemic blasts in three-color analysis. **Am J Clin Pathol 100**(5):534-40.

Carter RH, Spycher MO, Ng YC, Hoffman R, Fearon DT. 1988. Synergistic interaction between complement receptor type 2 and membrane IgM on B lymphocytes. **J Immunol. 141**(2):457-63.

Carter RH, Fearon DT. 1992. CD19: lowering the threshold for antigen receptor stimulation of B lymphocytes. **Science. 256**(5053):105-7.

Gabriel HH, Urhausen A, Valet G, Heidelbach U, Kindermann W. 1998. Overtraining and immune system: a prospective longitudinal study in endurance athletes. **Med Sci Sports Exerc 30**(7):1151-7.

Gane P, Fain O, Mansour I, Roquin H, Rouger P. 1992. Expression of CD11b (Leu15) antigen on CD3+, CD4+, CD8+, CD16+ peripheral llymphocytes. Estimation of CD3+8+11b+ and CD3+4-8-11b+ T-cell subsets using a single laser flow cytometer. **Scand J Immunol 36**(3):395-404.

Ginaldi L, Farahat N, Matutes E, De Martinis M, Morilla R, Catovsky D. 1996. Differential expression of T cell antigens in normal peripheral blood lymphocytes: a quantitative analysis by flow cytometry. **J Clin Pathol 49**(7):539-44.

Ginaldi L, De Martinis M, Modesti M, Loreto F, Corsi MP, Quaglino D. 2000. Immunophenotypical changes of T lymphocytes in the elderly. **Gerontology 46**(5):242-8.

Goyert SM, Ferrero E, Rettig WJ, Yenamandra AK, Obata F, Le Beau MM. 1988. The CD14 monocyte differentiation antigen maps to a region encoding growth factors and receptors. **Science 239**(4839):497-500.

Haziot A, Chen S, Ferrero E, Low MG, Silber R, Goyert SM. 1988. The monocyte differentiation antigen, CD14, is anchored to the cell membrane by a phosphatidylinositol linkage. **J Immunol 141**(2):547-52.

Heinzelmann M, Mercer-Jones M, Cheadle WG, Polk HC Jr. 1996. CD14 expression in injured patients correlates with outcome. **Ann Surg 224**(1):91-6.

Hultin LE, Matud JL, Giorgi JV. 1998. Quantitation of CD38 activation antigen expression on CD8+ T cells in HIV-1 infection using CD4 expression on CD4+ T lymphocytes as a biological calibrator. **Cytometry 33**:123-132.

Jason J, Archibald L, McDonald LC, Hart WM, Rheanppumikankit S, Tansuphwaswadikul S, Byrd MG, Larned J, Han A, Green TA, Jarvis WR. 1999. Immune determinants of organism and outcome in febrile hospitalized Thai patients with bloodstream infections.**Clin Diagn Lab Immunol 6**(1):73-8.

Lanier LL, Ruitenberg JJ, Phillips JH. 1986. Human CD3+ T lymphocytes that express neither CD4 nor CD8 antigens. **J Exp Med 164**(1):339-44.

Prince HE, York J, Jensen ER. 1992. Phenotypic comparison of the three populations of human lymphocytes defined by CD45RO and CD45RA expression. **Cell Immunol 145**(2):254-62.

Redelman D. 1987. Simultaneous increased expression of E-rosette receptor (CD2, T11) and T cell growth factor receptor on human T lymphocytes during activation **Cytometry. 8**(2):170-83.

Rickert RC, Rajewsky K, Roes J. 1995. Impairment of T-cell-dependent B-cell responses and B-1 cell development in CD19-deficient mice. **Nature. 376**(6538):352-5.

Roth MD. 1994. Interleukin 2 induces the expression of CD45RO and the memory phenotype by CD45RA+ peripheral blood lymphocytes **J Exp Med. 179**(3):857-64.

Sato S, Ono N, Steeber DA, Pisetsky DS, Tedder TF. 1996. CD19 regulates B lymphocyte signaling thresholds critical for the development of B-1 lineage cells and autoimmunity. **J Immunol. 157**(10):4371-8.

REPORT DOCUMENTATION PAGE

1. Report Date (DD MM YY) 14 Dec 00	2. Report Type New	3. DATES COVERED (from - to) Jan 2000 to Jan 2001

4. TITLE AND SUBTITLE
The Quantitative Immunophenotype (QuIP) is Characteristic of Individuals

5a. Contract Number:

5b. Grant Number:

5c. Program Element: 63706N

6. AUTHORS
Doug Redelman, Wayne Ensign & Donald E. Roberts

5d. Project Number: M0096

5e. Task Number: 004

5f. Work Unit Number: 6903

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Naval Health Research Center
P.O. Box 85122
San Diego, CA 92186-5122

5g. IRB Protocol Number:

9. PERFORMING ORGANIZATION REPORT NUMBER
Report No. 01-03

8. SPONSORING/MONITORING AGENCY NAMES(S) AND ADDRESS(ES)
Office of Naval Research
800 North Quincy St
Arlington, VA 22217-5600

Bureau of Medicine and Surgery
M2
2300 E St MW
Washington DC 02372-5300

10. Sponsor/Monitor's Acronyms(s)
BUMED

11. Sponsor/Monitor's Report Number(s)

12 DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

14. ABSTRACT (maximum 200 words)

The clusters of differentiation (CD) molecules used to identify lineages of cells often have important functional activity and the number of CD molecules expressed per cell can reflect the status of cell lineages. The quantitative immunophenotype (QuIP) , i.e., both the number of cells in various populations and the numbers of CD molecules expressed, can provide additional information about the functional capacity of the immune system. In order to examine the variations in QuIP among individuals and within individuals over time, blood samples from a cohort of Marines were tested three times over a two month period before and after various phases of strenuous advanced field training exercises. Samples were examined via a standard hematology analyzer to obtain complete blood counts and flow cytometrically with a panel of antibodies. The relative numbers of CD molecules per cell were estimated by using standard beads with known numbers of fluorochrome molecules per bead or with known antigen binding capacities per bead. Within individuals, the relative percentages of cell lineages and the numbers of CD molecules expressed per cell changed very little over the two months although there was substantial variation among individuals at each time. Thus, measureing the QuIP can provide additional and potentially valuable information about the status of the immune system.

15. SUBJECT TERMS
cluster differentiation molecules (CD); immunophenotype; flow cytometry; antibodies; lymphocytes, t-cells; monocytes; humans

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Commanding Officer
a. REPORT	b.ABSTRACT	b. THIS PAGE	UNCL	26	
UNCL	UNCL	UNCL			19b. TELEPHONE NUMBER (INCLUDING AREA CODE) COMM/DSN: (619) 553-8429

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39-18